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Alginate-hydroxyapatite Beads for Medical Application

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The paper describes the synthesis and characterization of alginate-hydroxyapatite (Alg/HA) beads that potentially can be used for drug release due to the adsorptive properties of HA and transmission ability of the soluble compounds through the alginate membrane. Calcium Alg/HA beads were manufactured by dropping of the Alg/HA suspension into $CaCl_2$ solution. Morphology and phase composition of obtained Alg/HA beads were investigated. It was shown that alginate macromolecules influence the hydroxyapatite size and morphology. Obtained HA with crystal size 15-30 nm inside alginate beads correspond a bioapatite size therefore are interesting for the tissue engineering. The main features of alginate-controlled crystallization are discussed in order to understand some aspects in composite material design. Insertion of HA into the alginate microcapsules allows use such materials for production of drug release microspheres.

Keywords: Alginate, Hydroxyapatite, Microspheres, Beads.

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1. INTRODUCTION

Last years a great attention has been paid to the polysaccharide-based hydrogel beads obtained through ionotropic gelation technique. They can be used as potential carriers in controlled drug delivery [1]. The use of alginate microspheres is useful in controlling the release rate of low molecular weight drugs [2]. Sodium alginate, a hydrophilic anionic carbohydrate biopolymer composed of varying proportions of d –mannuronic acid (M) and l – guluronic acid (G) residues which are arranged in MM or GG blocks interspersed with MG blocks [3]. It has unique property of forming water insoluble calcium alginate gel through gelation with divalent calcium (Ca^{2+}) ions in a simple and mild condition made possible to encapsulate both macromolecular and low molecular agents in calcium alginate beads [4]. Hydroxyapatite (HA) is the main inorganic component of vertebrate hard tissues such as bones and teeth is widely used due to its bioactivity, high bio-compatibility and stability and its adsorption properties [5]. The as-prepared beads are characterized by X-ray powder diffraction (XRD) and optical electron microscopy.

2. MATERIALS AND METHODS

2.1 Materials

Sodium alginate (Alg), $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ powders and 25 % ammonia solution were of analytical grade and used without further purification. Sodium alginate powder was diluted in distilled water and the solution was agitated for 24 h to obtain homogeneous solutions with concentrations of 1 % and 3 % (w/v) of Alg respectively.

Synthesis of Alg/HA microspheres contains two stages. At the first stage hydroxyapatite hydrogel was synthesized according to the following reaction:

$$10Ca(NO_3)_2 + 6\,(NH_4)_2HPO_4 + 8\,NH_3{\cdot}H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20\,NH_4NO_3 + 6\,H_2O \quad (1)$$

It was washed with following centrifugation and mixed with 3 % (a) or 1 % (b) sodium alginate solution respectively. Obtained suspensions were dripped into 0.1 M $CaCl_2$ (200 mL) solution. These granules were kept in 0.1 M solution of $CaCl_2$ overnight for the final conformation of the polymer. Owing to interaction of alginate macromolecules with Ca^{2+} ions in aqueous solution alginate microspheres with inserted hydroxyapatite is formed. The formed Alg/HA beads were filtered out and washed with distilled water for three times. Than obtained beads were placed in air overnight and dried in an oven at 37°C for 48 h.

2.2 Methods

Powder XRD analyses were performed using a diffractometer (DRON-3, Bourevesnik, Russia), CuKα radiation (λ = 0.154 nm), running at 40 kV and 20 mA, scanning from 10°to 60° at 4°/min. Identification of crystal phases was provided by card catalog JCPDS (Joint Committee on Powder Diffraction Standards). Crystal sizes were calculated by using Scherer's formula. Surface morphology was visualized by optical microscopy (Primo star, Karl Zeiss Group).

3. RESULTS AND DISCUSSION

3.1 Morphology of Alg/HA beads

Morphology of Alg/HA microspheres are presented in Fig. 1 and Fig. 2.

The average size of 3 % Alg/HA microspheres is 1600 μm and 1300 μm for 1 % Alg/HA microspheres. The average size could be varied by changing of dropper nozzle size and viscosity of the initial solution.

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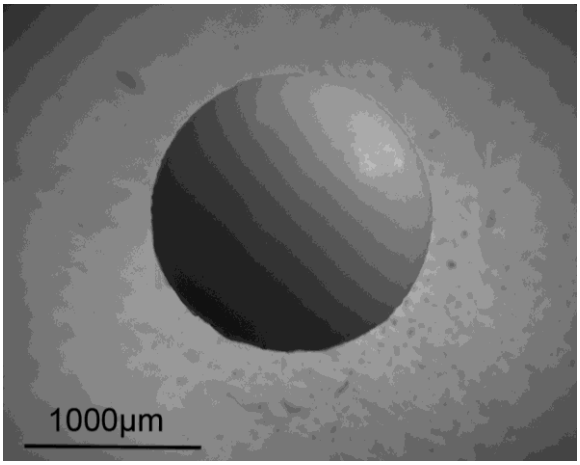

Fig. **1** – Morphology of the Alg/HA microsphere obtained from 3% sodium alginate solution

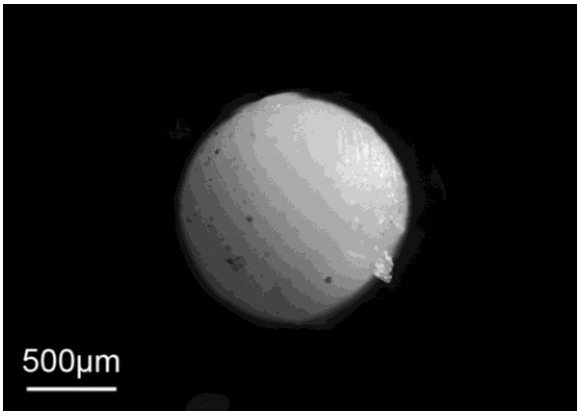

Fig. 2 – Morphology of the Alg/HA microsphere obtained from 1 % sodium alginate solution

Composite Alg/HA beads have spherical shape that is easy to reproduce and effectively used in mineralization of bone tissue. Besides, such microspheres can be loaded with drugs and necessary protein systems (growth factors, ets.)

3.2 XRD study

Fig. 3 illustrates the XRD patterns of 1 % Alg/HA (a) and 3 % Alg/HA (b). The diffraction peak of sodium alginate at around 13.47° is a typical characteristic peak of the hydrated crystalline structure. The main crystalline phase is hydroxyapatite. The average crystal sizes that

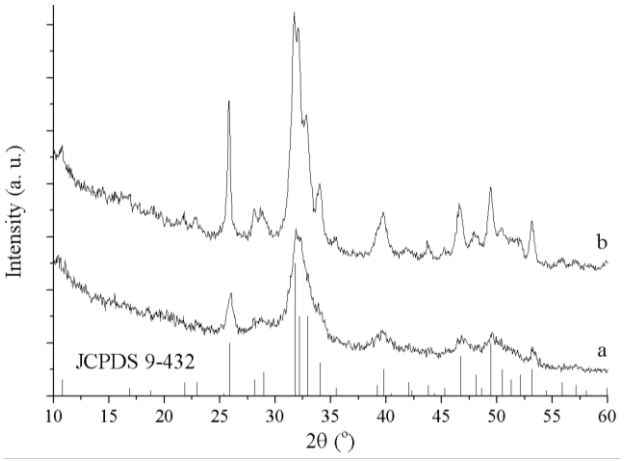

Fig. 3 – XRD patterns of the Alg/HA microspheres obtained from 1% (a) and 3% (b) sodium alginate solutions mixed with HA hydrogel

were calculated using Scherer's equation for (002) planes are 15-30 nm.
The nano-sized crystals of HA (15-30 nm) are homogeneously distributed within the alginate beads, which is expected to modify the mechanical properties of the gel network.

Swelling ratio S_t at a given time of obtained Alg/HA beads were calculated using the following equation:

$$S_t = \frac{(m_t - m_0)}{m},$$

where m_0 and m_t are the weights of the dry sample and the swollen sample, respectively. S_t was calculated as g of water per gram of sample. For 1 % Alg/HA beads and 3 % Alg/HA beads swelling ratio were 293 %, 153 % and porosity 38 %, 35,7 % respectively.

4. CONCLUSIONS

In this work the inorganic phase (HA) is formed within polysahharide alginate matrix that mimics creation of structures close to natural nanocomposites. The HA nano-particles act as inorganic crosslinkers in the nano-composites, which could decrease the movability of the alginate polymer chains, change the surface morphology and decrease the swelling ratio. This method may be applied in the preparation of new complicated beads for the controlled delivery of drugs.

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